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The major objective of this proposal was to investigate whether somatostatin (SRIF) may be a non-steriod inhibitor of ACTH release. The goals of the proposal were to examine the structure of the SRIF receptor and determine whether glucocorticoids regulate the expression of the SRIF receptor in pituitary. In our previous reporting period, we showed that glucocorticoid downregulate the pituitary SRIF receptor. This downregulation could involve a sequestration of the receptor or an inhibition of the synthesis of SRIF receptor. To be able to examine the synthesis and expression of the SRIF receptor, we are presently trying to clone the gene encoding the SRIF receptor. This is being attempting using two approaches. First we have purified the receptor to homogeneity and are attempting to sequence it. Second, we were recently able to express the receptor in oocytes and are using this capability for expression cloning of the receptor. Furthermore, we have been able to demonstrate the functionally distinct subtypes of SRIF are expressed. One receptor preferentially binds SRIF-14 while the other binds to SRIF-28. Attempts are underway to determine the molecular basis for these SRIF receptor subtypes.

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ANNUAL REPORT ON CONTRACT ONR N00014-88-K-0048

PRINCIPAL INVESTIGATOR Terry Reisine

CONTRACT TITLE: Inhibition of ACTH release by peptide hormones: Molecular mechanisms and possible role as anti-stress factors.

Contract Period: Nov. 1, 1988 to Oct, 30, 1989.

INTRODUCTION AND RESEARCH OBJECTIVES: The major objective of the research proposal was the identification of non-steriodal factors which inhibit ACTH release and may be useful in the treatment of stress. Previously, it was reported that the hypothalamic peptide somatostatin (SRIF) could inhibit CRF stimulated ACTH release from a tumor cell line of the anterior pituitary, AtT-20, which consists of a homogeneous population of ACTH secreting cells. While administration of SRIF to non-stressed, humans failed to alter plasma ACTH or cortisol levels, patients with adrenal insufficiency did respond to SRIF with a lowering of ACTH release. This latter finding, may indicate that SRIF has some role in the suppression of ACTH secretion in humans but that under normal conditions, glucocorticoids downregulate or diminish the effectiveness of SRIF in regulating ACTH secretion. In fact, several studies have suggested that glucocorticoids downregulate SRIF receptors in the anterior pituitary. Furthermore, Lamberts et al (Endocrinol. 118:2188, 1986), showed that SRIF could inhibit CRF stimulated ACTH release from anterior pituitary cells in culture grown in the presence of glucocorticoid antagonists such as RU 486. Thus, a major aspect of the proposal was to determine the mechanisms by which SRIF may regulate ACTH release and whether this effect was in fact tonically suppressed by glucocorticoids.

Three aspects of the proposal for which we have gained substantial progress since the start of funding are; 1) the structural analysis of the SRIF receptor, 2) the effect of glucocorticoid treatment on SRIF receptor expression; and 3) the structural analysis of SRIF receptor subtypes.

PROGRESS REPORT;

1. Physical properties of the SRIF receptor: As indicated in our previous report, we have been able to purify the SRIF receptor to near homogeneity (He et al., 1989). We have now made several attempts to sequence the receptor. In our attempts we have learned that the receptor in N-terminally blocked. This indicates that it is necessary to digest the receptor in order to generate fragments that can then be sequenced. We found that cyanogen bromide is capable of digesting the receptor. Presently we are purifying enough of the receptor so that we can digest the protein and sequence the fragments.

We have also been able to approach the cloning of the SRIF receptor using a different approach than sequencing the receptor. We have been able to express the receptor in oocytes following injection of mRNA extracted from the cell line AtT-20 which has a high expression of SRIF receptors (White and Reisine, in press). The SRIF receptors that are expressed in the oocytes are functionally active and can mediate SRIF's inhibition of adenylate cyclase activity in individual oocytes. Using this approach, we can screen clones from an AtT-20 cell cDNA library for expression of the SRIF receptor. These cloning studies are now in progress.

In addition to the studies on the purified SRIF receptor, we have recently been able to solubilize the SRIF receptor in an active form and detect the receptor using receptor binding techniques (He et al., submitted). The receptor from brain or AtT-20 cell was solubilized with the detergent CHAPS and the receptors were labeled with [125I] MK 678, a highly selective SRIF agonist. The solubilized SRIF receptor retains the same characteristics as the membrane bound receptor. Furthermore, the solubilized SRIF receptor is tightly associated with GTP

binding proteins. We are in the process of determining whether subtypes of SRIF-14 and SRIF-28 receptors are coupled to different GTP binding proteins. This will be done by immunoprecipitating the solubilized receptors from GH_3 and AtT-20 cells that are complexed with GTP binding proteins with antibodies selective for different species of the GTP binding proteins. These studies may allow us to determine whether subtypes of SRIF receptors may mediate different functions of SRIF by coupling to different GTP binding proteins.

Finally, in order to clearly establish the subtypes of SRIF receptors exist, we have performed electrophysiological studies in brain neurons and tested the effects of SRIF-14 and SRIF-28 on K^+ currents (Wang et al., in press). SRIF-14 increases the K^+ current in a concentration dependent manner that was prevented by pertussis toxin which suggests that SRIF-14 receptors coupled to K^+ channels via GTP binding proteins. SRIF-28 inhibited the same K^+ current in a concentration dependent manner that was also prevented by pertussis toxin. SRIF-14 and SRIF-28 induced opposite effects on the K^+ current in the same neurons suggesting the these peptides act through different receptors to regulate this K^+ current in brain neurons. This is the first evidence that SRIF-14 and SRIF-28 induce different actions in the brain.

EXPENDITURES

Supplies: expenditures normal

Equipment: none.

Travel: Travel funds were used by the PI and Henry Wang to attend the Soc. for Neuroscience Meeting in Phoenix.

Personnel: Henry Wang: Research Assistant (100% support).

Women or minorities- 0; Non-citizens - 1.

Publications

Abstracts:

Wang, H., Reisine, T. and Dichter, M.: Somatostatin-14 and Somatostatin-28 inhibit Ca^{++} currents in rat neocortical neurons. Soc. Neurosci. Abst 15:217.

White, M. and Reisine, T.: Expression of Functional pituitary somatostatin receptor in Xenopus ooctyes. Soc. Neurosci. Abst. 15:345.

Raynor, K. and Reisine, T.: Cyclic analogs of somatostatin distinguish pharmacologicall and functionally distinct subtypes of somatostatin receptors in brain and pituitary. Soc. Neurosci. Abst. 15:990.

He, H.T., Rens-Domiano, S., Borislow, S. and Reisine, T.: Biochemical properties of the solublized brain somatostatin receptor. Soc. Neurosci. Abst. 15:990.

Manuscripts:

Reisine, T.: Phorbol esters and corticotropin releasing factor stimulate calcium influx in the anterior pituitary tumor cell line AtT-20 through different intracellular sites of action. J. Pharmacol. Expt. Therap. 248:984-990, 1989.

He, H.T., Johnson, K., Thermos, K. and Reisine, T.: Purification of a putative brain somatostatin receptor. Proc. Natl. Acad. Sci. 86:1480-1484, 1989.

Thermos, K., He, H.T., Wang, H., Margolis, N. and Reisine, T.: Biochemical properties of brain somatostatin receptors. Neuroscience 31:131-141, 1989.

Wang, H.L., Bogen, C., Reisine, T. and Dichter, M.: Somatostatin-14 and Somatostatin-28 induce opposite effects on potassium currents in rat neocortical neurons. Proc. Natl. Acad. Sci. in press.

White, M. and Reisine, T.: Expression of functional pituitary somatostatin receptors in Xenopus oocytes. Proc. Natl. Acad. Sci. in press.

Raynor, K. and Reisine, T.: Analogs of somatostatin selectively label distinct subtypes of somatostatin receptors in rat brain. J. Pharmacol. Expt. Therap. in press.

Reisine, T.: Pertussis toxin in the analysis of receptor mediated signal transduction. Biochem. Pharmacol. in press.

3ND YEAR WORK PLAN: The objectives of year 2 of funding are 1) to obtain partial sequence information of the purified SRIF receptor to begin cloning of the gene coding for this protein; 2) to determine what are the structural differences in SRIF receptor subtypes in AtT-20 and GH3 cells, and 3) to further characterize the functional differences of subtypes of somatostatin receptors. .